TUBERCULINS AND THE TUBERCULO-OPSONIC INDEX.*

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THE present paper is in no sense a review of the work done and results achieved in this interesting field. My aim is to present the subject to you as it has presented itself to me. I shall be thoroughly practical and shall discuss chiefly the method of determining the tuberculo-opsonic index as I am practising it, and the tuberculins.

In the *Deutsche medizinische Wochenschrift* for April 1, 1897, Koch described his improved tuberculin. He told in what way the old tuberculin had disappointed, that repeated injections of it were followed by repeated improvements, but that these improvements had a limit, for an immunity was established against the tuberculin itself. This immunity may last several months. This immunity has no influence on the tubercle bacilli; it is, therefore, purely a toxin-immunity, not a bacterial one. The power to react was, unfortunately, often lost before a complete cure was established. A return of this power of reaction had to be awaited before the treatment could be continued. Where, however, sufficient patience and skill were exercised many cases might be cured, and he would still have felt that in the old tuberculin we had the best remedy for tuberculosis if it had not proved possible from tubercle cultures to make a pure bacterial-immunizing preparation. This, to be brief, he prepared as follows: The tubercle bacilli from young virulent cultures were desiccated in vacuo, ground to pieces in a mortar, taken up in distilled water and the mixture centrifugalized. There resulted an upper slightly opalescent fluid and a sticky sediment. The sediment was dried and put through the above process again. The resulting sediment was again treated in the same way, and so on until only incidental dirt remained as a sediment. The first supernatant nearly clear fluid he called Tuberculin O (oberste Schicht), or, for short, TO. This he found had properties very like those of the old tuberculin. The subsequent fluids resulting from the grinding and other manipulations of the successive sediments were mixed together and named Tuberculin R (Rests), or, for short, TR. It was so made that 1 c.c. contained 10 mg of solid substance. This solid consisted of pulverized tubercle bacilli minus certain elements which had been extracted and were contained in the TO. A peculiarity of TR which distinguished it from the old tuberculin and from TO was that its injection was to a far less degree followed by a temperature reaction. Koch regarded this as a distinct advantage possessed by TR over TO and old tuberculin. Koch began with injections of $^1/_{500}$ mg. of TR and gradually raised the dose to usually 20 mg. These figures refer to the solid substance in the TR. Secondary symptoms to cause anxiety, or any other encroachments on health which could be ascribed to the preparation, Koch said he saw in no case. Especially striking was the progressive straightening out and return to normal of the temperature curve. He said he did not venture to maintain that the method of beginning with minimal injections and gradually raising them to 20 mg. was the best way of using TR. He thought other methods of procedure, perhaps even combinations of TR with TO or with sera obtained with TO or TR might be found to be more effective. But further improvement in the matter of preparations made from tubercle cultures he did not think was to be looked for.

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Another important communication from Koch appears in the same periodical under date of November 28, 1901. The subject of agglutination of the tubercle bacillus by sera of patients had come up. Koch made an exhaustive study of it. Before this there had been no means of measuring the degree of immunity attained. Koch came to the conclusion that the agglutination test gave this needed measure. In earlier immunizing attempts he had given to TR the preference over

TO because TR gave relatively weaker reactions than TO. But, guided by the agglutination test he now found it better to use the undivided culture mass, and, further, that the agglutinating power in man as well as in animals developed most surely and quickly when the reactions were not too feeble and when the doses were raised as rapidly as possible. He made a new tuberculin containing one part of pulverized tubercle bacilli in 100 parts each of water and glycerine. One c.c. thus represents 5 mg. He began with injections of 0.0025 mg. and rapidly increased the dose. He found that when agglutination had set in, or when that originally present had been increased, it was important to continue raising the dose; otherwise the agglutinating power would sink. He went as high as 20 mg. and in some cases 30 mg. That the development of agglutinating power is actually associated with the formation of protective substances he felt could be inferred, because from the moment that agglutination was established in his phthisis cases the condition of the patients was distinctly better. Some patients failed to develop agglutination, and in them of course the agglutination test could not be used to control the treatment. The preparation which Koch used for his injections is known as " New Tuberculin Koch (Bacilli Emulsion)." Both it and TR, which is now labelled " New Tuberculin Koch (TR)," are manufactured by the Farbwerke vorm. Meister Lucius and Brüning and are imported into this country by their agents, Victor Koechl and Co., 122 Hudson Street, New York.

Koch felt that the agglutination test gave him a measure of the degree of immunity which his tuberculin was producing. Wright believes he has discovered a better measure in the opsonic index. He infers from experiments that there are substances in the blood which act upon bacteria to make them easier victims for the phagocytic leukocytes. These substances he calls opsonins. There are apparently various opsonins for the various microörganisms. The proportion of opsonin in the blood Wright takes to be the measure, if not the reality, of the immunity. The relative amounts of opsonin in the blood of a healthy individual and in that of a patient are determined by observing the phagocytic powers of leukocytes when in the presence of bacteria acted upon by the serum of the patient, and their phagocytic powers when the bacteria are acted upon by the serum of a healthy individual. The average number of bacteria found in a polynuclear leukocyte in the case of the patient's serum, divided by the average number found in the case of the healthy individual's serum, is the opsonic index of the patient. It is expressed in decimals for convenience of comparing various indices.

I have chiefly concerned myself with the tubercular index. In spite of reading the contributions of Wright, I have found this work difficult. Dr. Simon Flexner has saved me much preliminary stumbling by showing me the opsonic work which Dr. Edwin H. Shorer has been engaged in at the Rockefeller Institute. I am deeply indebted to Dr. Flexner and Dr. Shorer for introducing me to the practical details of opsonic technique.

As the communications of Wright are accessible to all, I shall not take your time to describe the mode of determining the tuberculo-opsonic index in all its many details, but shall confine myself to speaking of the snags which I have encountered and the ways in which I have put them aside. The three ingredients for determining the phagocytosis are the serum, the mixture containing the leukocytes, and the suspension of tubercle bacilli. There is no trouble about getting the serum. The only point about the leukocytes is to centrifugalize them at such a slow rate that they shall not be pressed together into coherent clusters, but shall remain discrete. I find that if the centrifuge runs at 900 revolutions for half an hour the result is perfect. There is more trouble about the suspension of tubercle bacilli. For making this I have used the coarse powder of agglomerated bacilli which is a by-product in the manufacture of tuberculin. The method of grinding this in a mortar results in a considerable fragmentation of bacilli, which adds somewhat to the difficulty of counting accurately, and the grinding is tedious. I have discarded the mortar and have made a shaking machine which I use instead. It contains a mill-wheel run by water from an ordinary faucet. To the wheel-shaft is attached a crank, and to the crank a rod, the further end of which plays through a bearing about 14 inches distant. To this rod is attached a trough, into which the object to be shaken is snugly packed in cotton and firmly tied. The stroke is 2 inches and the crank makes about 400 revolutions a minute. I put some of the bacilli powder, some glass beads and the suspension fluid into a glass tube and seal the tube. I put this tube in the shaker, turn on the water and let the machine run six or eight hours. Individual bacilli become disengaged in abundance and there are few fragments. I then fill a tube for two inches with the opaque shaken mixture and centrifugalize it at about 3,000 revolutions for six minutes, after which the top half inch is aspirated off. This fluid may contain too many bacilli, in which case the proper dilution is made.

Another difficulty I have found in getting the smears well stained for counting purposes. There are three desiderata. The bacilli should be intensely stained, so as to be seen with the least effort. The nuclei should be lightly stained, so that bacilli overlying them are readily seen. The outlines of the leukocytes should be visible.

I have struggled much over this matter and have arrived at the following staining method: Fix 20 seconds in wood alcohol. Wash. Stain 5 to 10 minutes in a bath of carbol-fuchsin, standing in boiling water. (I have made the carbol-fuchsin of double strength, 20 parts sat. alc. sol. fuchsin to 80 parts 5 per cent. carbolic acid, and evaporated it down on a water bath until it was of sufficient strength to make a good reddening of the smear in 5 minutes.) Wash. Decolorize one minute in 5 per cent. H_2SO_4. Wash. Counterstain 30 seconds in ¼ per cent. sat. alc. sol. methylene blue in ½ per cent. sol. Na_2CO_3. Wash lightly. Dry by agitation in the air. If the smears are good, the pictures are also good.

I wish I felt justified in saying something about results of treating cases of surgical tuberculosis under control of the opsonic index. I have encountered so many difficulties in the technique that my energies have thus far been mostly absorbed in trying to overcome these. In this field it is folly to make clinical deductions until one is sure of getting indices of fairly uniform accuracy. As the sera of patients are supposed to vary widely without treatment and to fluctuate under the influence of treatment, the accuracy of the determination can best be gauged, it seems to me, by including in each batch of sera to be opsonized, the sera from more than one normal individual. These normal sera serve as opsonic standards in the same way that a pasted serum does, and in addition they serve as mutual controls upon the accuracy of the work. If the indices of the normal sera are not in accord, suspicion is cast upon the results in the whole series. I have adopted this method of control, using my own serum and those of my two sons. My results are as yet too few to warrant definite conclusions as to the accuracy of the technique or the value of the tubercular index, but my experience makes me feel that the results obtained by anyone who has not thus controlled his work until quite sure of his ground are to be received with scepticism.

Wright has used for his injections Koch's TR, and has generally begun with a dose of $^1/_{1000}$ mg. He has usually found that the index could be kept highest by using very small doses, rarely going higher than twice or three times this amount. In the matter of dosage he is thus at great variance with the conclusions reached by Koch when he controlled the dosage by the agglutination test. It would seem that if the opsonic index is the true criterion of dose and interval, then the agglutination test is not the true criterion. It is a question whether the Bacilli Emulsion is or is not better than TR in this treatment. The whole subject calls for much careful work with the closest coöperation between the clinic and the laboratory.

The brilliant results recorded by Wright and his colleagues compel enthusiastic attention to the subject, intricate and laborious though its demands are. Only through careful work by many impartial observers can its practical usefulness be shown. If others can achieve the results which Wright and his fellow-workers record we shall be able to treat effectively cases of surgical tuberculosis in which operative treatment and other surgical measures now leave so very much to be desired.